Modeling Arenavirus Nucleocapsid and Z Protein Structures

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Abstract:

The Arenaviridae family of viruses, responsible for neurological disease and hemorrhagic fever, is transmitted to humans via rodents. Over 20 different strains have been identified and phylogenetically classified since the first outbreak of the virus in 1933 and new strains are continuing to develop. It is known that Arenaviridae are enveloped and spherical, meaning that they are made up of a single protein copied numerous times, and contain two segments of single stranded RNA [1]. There is no structural information about the major nucleocapsid protein (NP) and other proteins associated with the virus capsid structure. This lack of structural data has hindered the identification of potential drug targets and the development of effective drugs. Currently there are no vaccines or FDA approved drugs for Arenavirus infections.

The zinc-finger-like protein (Z) is known to interact with NP to induce budding, the process of viral proliferation. In order to better understand how NPs interact in the context of the spherical virus shells as well as with Z, structures of these two proteins were predicted using homology modeling methods. Amino acid sequences for the Old World Lymphocytic Choriomeningitis Virus (LCMV) and the New World Tacaribe Virus, two strains commonly studied in experimental labs, were used for prediction of tertiary structure. Models for Z were constructed from templates obtained through PSI-Blast and compared to the tertiary structure predictions from web servers. In addition, tertiary structures of all known virus capsids described in Viper DB [2] were used as potential templates for homology modeling of NP. Having constructed models of NP and Z, we identified putative protein-protein interaction sites, which may represent a better anti-viral drug target than the interaction of Z with human proteins.

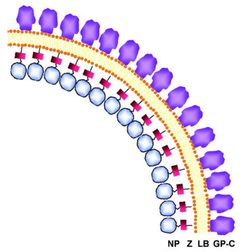
[1] Neuman, B.W., B.D. Adair, J.W. Burns, R.A. Milligan, M.J. Buchmeier, M. Yeager, Complementarity in the Supramolecular Design of Arenaviruses and Retroviruses Revealed by Electron Cryomicroscopy and Image Analysis, *J. Virol.* 2005, Vol. 79, 3822-3830

[2] Shephed, C.M., I.A. Borelli. G. Lander. P. Natarajan, V. Siddavanhalli, C. Bajaj, J.E. Johnson, C.L. Brooks, V.S. Reddy, VIPERdb: a relational database for structural virology, *Nucleic Acids Res.* 2006, Vol. 34, D386-D389

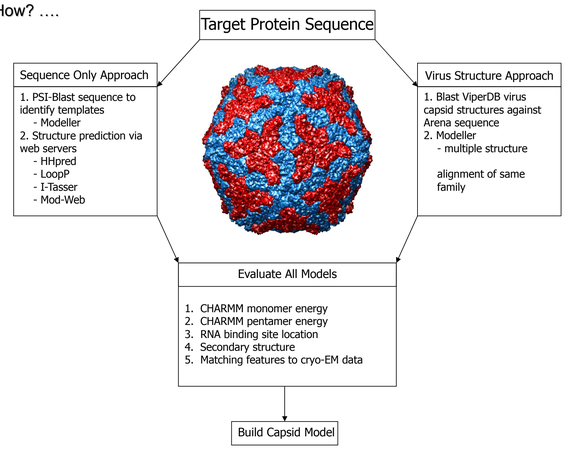
Objectives/Methods:

What?
- Create models for NP and Z proteins

Why?
- No structural information
- No Vaccines
- No FDA approved drugs
- Potential to design new drugs
  - Z protein is major drug target

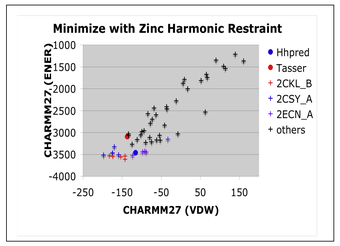


How? ....



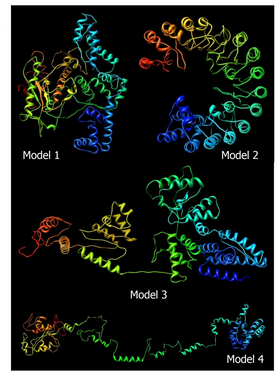
Z Protein: Sequence Only Approach

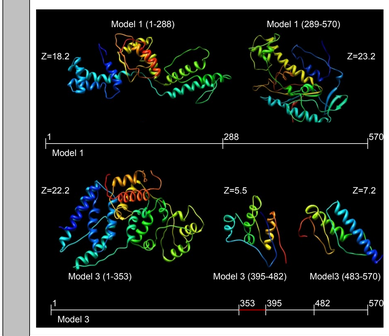
- Zinc-finger motif in sequence
- PSI-Blast provided 10 templates
  - E-value
  - Percent identity (~30%)
- Model criteria: correct zinc binding geometry
  - Modeller allows for incorporation of ligand geometry
- Evaluated CHARMM energies
- Models converged to a favorable fold
- All models were within 1.5-2.4 Å RMSD



NP: Sequence Only Approach

- HHpred, Mod-Web and LoopP produced poor structures - unfolded/unreasonable
- Only I-Tasser gave full-length, folded and well-packed models
  - no viral protein templates
  - some models had favorable CHARMM energies
  - other models were unreasonable
- Leucine Rich Repeat (LRR) domain
  - helical repeat protein
  - unlikely to be a capsid protein
  - poor CHARMM energy

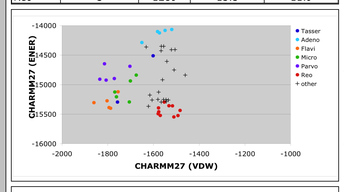


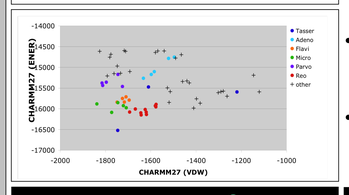


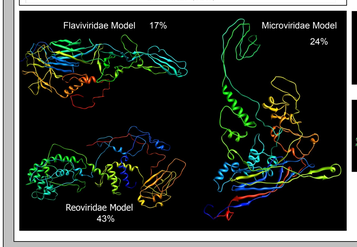
- I-Tasser models broke down into separate domains
- Individual domains were submitted to DALI
- DALI Z scores indicate that these domains occur in other protein structures
- Domains are candidates if virus space hypothesis is incorrect

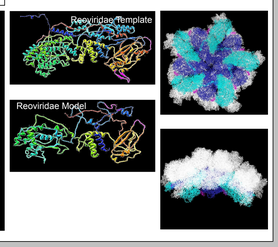
NP: Virus Structure Approach

	N templates	Length	% ID (LCMV)	% ID (TAC)
Adeno	2	940	10.2	10.5
Flavi	3	495	11.8	14.3
Micro	4	426	12.2	13.1
Noda	3	361	19.2	20.0
Parvo	5	579	10	9.0
Picorno	4	300	14.4	14.0
Polyoma	3	380	16.1	17.8
Reo	3	1289	13.3	11.9







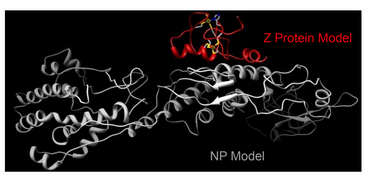


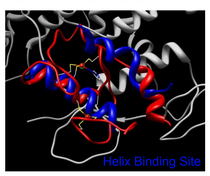
- Ran Blast of Arenavirus against all of ViperDB
- Eight virus families were identified as potential templates
- Built models using multiple structure alignment from same family
- Monomer energies were evaluated using CHARMM27 minimization with implicit solvent model
- Predicted secondary structure was compared to the secondary structure in the model
- BindN was used to predict strongest RNA binding site on Reoviridae template and model
- RNA binding sites were found on the inside of the capsid in a reasonable location

Comparison of Z and NP Modeling

Z Protein (90 residues)	NP (570 residues)
• 10 templates from PSI-Blast	• No suitable templates from PSI-Blast
• Multiple methods converged on similar structure	• No consensus between web server predictions and Modeller
• LCMV and Tacaribe shared best template (2CKL_B)	• Restricted search templates to virus structures
• High confidence in prediction	• LCMV and Tacaribe shared best virus template (Reoviridae)
	• Moderate confidence in prediction

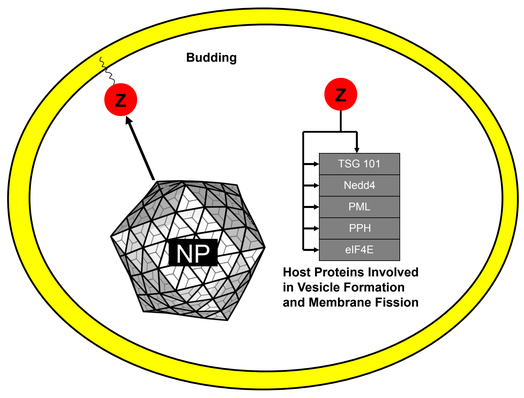
Prediction of Z and NP Protein-Protein Interaction

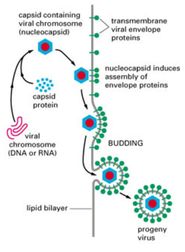




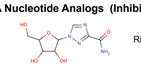
- Protein-protein interactions were inferred from protein binding on Reoviridae inner shell
- Z protein was matched to putative helix-binding site

Interaction of Z and NP



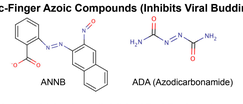


RNA Nucleotide Analogs (Inhibits RNA Replication)



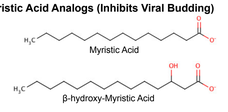
Ribavirin

Zinc-Finger Azoic Compounds (Inhibits Viral Budding)



ANNB ADA (Azodicarbonamide)

Myristic Acid Analogs (Inhibits Viral Budding)



Myristic Acid

β-hydroxy-Myristic Acid

Conclusions/Future Directions

- High confidence in Z protein modeling
- Models based on Reoviridae templates are better than other virus-based models based on evaluation criteria
- Continue working on NP-NP interactions to build full capsid
- Improve upon models of Z binding to NP
- Further work could focus on drug development to inhibit Z and NP interaction to inhibit budding
- Possibility of using I-Tasser domains to build novel Arenavirus fold